PROCEEDINGS
OF THE
NATIONAL ACADEMY OF SCIENCES
Volume 30 April 15, 1944 Number 4
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THE BEHAVIOR OF OPPOSITIONAL ALLELES IN POLYPLOIDS OF TRIFOLIUM REPENS[1]

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Communicated February 16, 1944

The theory of oppositional alleles was first proposed with substantiating data by East and Mangelsdorf[2] to explain self- and cross-incompatibilities in *Nicotiana*, and subsequently it was confirmed by other workers in several diploid species. Corollary hypotheses have been proposed to extend its application to polyploids, but because of the paucity of experimental data no hypothesis has been verified completely. The colchicine technique, which allows production of polyploids from plants of known genotype, should provide a more precise approach to this problem, but the results published so far have been conflicting. Stout and Chandler[3] reported that 15 diploid plants of *Petunia axillaris* were self-incompatible whereas tetraploid branches induced on the same plants were self-compatible. The same authors[4] showed that the 212 seedlings obtained from the tetraploid branches were likewise self-compatible and within families were cross-compatible. A similar situation was described by Crane and Lewis[5] for pears wherein most of the 29 varieties tested were self-incompatible or of low self-compatibility. The exceptions were "Fertility 2 ×," which sets fruits but no seeds, and "Fertility 4 ×," which sets both fruits and seeds. In contrast with these results, Howard[6] reported that both types of branches on diploid-tetraploid sectorial chimeras in *Brassica rapa*, *B. campestris* and *Raphanus sativus* have always been self-incompatible. Likewise Lewis[7] found the autotetraploid branches of *Oenothera organensis* to be self-incompatible like the diploid; the inhibition of pollen tubes, which was strong in the diploid, was reduced in the tetraploid but not enough to allow fertilization. In extensive tests of autotetraploid maize, Randolph[8] and Fischer[9] observed that different stocks varied from low to high self-fertility and that these differences were accounted for only in part by cytological irregularities. Certain self-sterile stocks were ordinarily cross-sterile as females but cross-fertile as males. There was also some

evidence of cross-incompatibilities between certain self-fertile stocks. Since genes for incompatibility have not been described for diploid maize, Fischer[9] suggested that chromosome doubling results in a change in genic balance due to the cumulative action of some genes and the non-cumulative action of others.

Because suitable terms to describe multiple alleles in autotetraploids would facilitate the discussion, the following new terminology is suggested and will be used in this paper:

NO. OF DIFFERENT ALLELES	EXAMPLE	SUGGESTED TERM		DESIGNATION IN TABLE I
None	$S_1S_1S_1S_1$	Monoallelic		...
Two	$S_1S_1S_1S_2$	Unbalanced	Diallelic	...
Two	$S_1S_1S_2S_2$	Balanced	Diallelic	II
Three	$S_1S_1S_2S_3$	Triallelic		III
Four	$S_1S_2S_3S_4$	Tetraallelic		IV

As a result of treating white clover (*Trifolium repens* L.) seedlings with colchicine,[10] several plants were obtained on which some of the stolons had the doubled number of 64 chromosomes, whereas other stolons on the same plants retained the original number of 32. When preliminary tests of these paired sectors in the greenhouse and in the field indicated that both 32- and 64-chromosome parts were self-incompatible, six of the pairs were tested more extensively in the greenhouse. The techniques used for selfing and crossing were the same as those described previously.[11] Chromosome counts from root tips of the new cuttings showed that the 32 and 64 numbers had been maintained in each case. A rapid examination of the pollen was made with a hand lens, and every plant was found to produce a very high percentage of apparently normal, well-filled pollen. In this repeat test, both 32- and 64-chromosome cuttings again proved self-incompatible. The 32-chromosome slips yielded an average of 0.11 seed per head on the 35 heads that were self-pollinated, and the 64-chromosome slips yielded 0 seeds on 30 heads. All 61 crosses that were tried between different 32-chromosome cuttings were compatible and averaged 34.1 seeds per head. Likewise, all 31 crosses between 64-chromosome cuttings proved compatible, but they averaged only 17.5 seeds per head. The difference between these two averages was highly significant. With the 38 heads used in 64 × 32 crosses, however, only 0.29 seed per head was obtained, and with the 67 heads used in 32 × 64 crosses the average seed set was 0.51.

On the basis of these results, two 32-chromosome cuttings and the corresponding two 64-chromosome cuttings from the same two original seedlings were selected for a test of the compatibility relationships of their F_1 populations. In the 32 × 32 family, 13 F_1 plants consisted of four intra-

sterile, inter-fertile groups of four, four, three and two plants, respectively, and all were reciprocally compatible with both parents. Among the 178 compatible crosses, the highest seed set per head was 56 and the lowest 23 with an average of 42.2. The differences in average seed set between the plants as females were highly significant in two of the groups (those with four plants each), but not significant in the other two. Among the same plants as males, there were no significant differences in average seed set. The maximum seed set among the incompatible crosses was 5, the minimum 0, and the average for the 35 heads (three crosses were made in duplicate) was only 0.37. The two parent plants proved self-incompatible, and the 13 F_1 plants reacted the same way. When three heads on each of the two parents were tripped to effect self-pollination, using 10 flowers per head, 0 seeds were obtained on the female and only one on the male. Likewise 0 seeds were obtained when 5 heads on each of the parents were manipulated. Similarly, the 13 F_1 plants yielded a maximum of one seed per head and an average of 0.13 when three heads per plant were tripped and an average of only 0.90 when a total of 73 heads were manipulated.

This type of self- and cross-incompatibility is explained by assuming that a single series of oppositional alleles conditions pollen-tube growth and that in this case the parents had no alleles in common:

$$S_1S_2 \times S_3S_4 = S_1S_3 + S_1S_4 + S_2S_3 + S_2S_4$$

When sectors of the 32-chromosome parent plants were doubled, the oppositional alleles likewise would have been doubled:

$$S_1S_1S_2S_2 \text{ and } S_3S_3S_4S_4$$

A cross between two such cuttings should yield the following genotypes in the F_1 generation:

DIALLELIC	TRIALLELIC	TETRAALLELIC
1 $S_1S_1S_3S_3$	4 $S_1S_1S_3S_4$	16 $S_1S_2S_3S_4$
1 $S_1S_1S_4S_4$	4 $S_2S_2S_3S_4$	
1 $S_2S_2S_3S_3$	4 $S_1S_2S_3S_3$	
1 $S_2S_2S_4S_4$	4 $S_1S_2S_4S_4$	

The frequency shown is based on random 2 × 2 assortment of the four homologous chromosomes with no crossing-over between the S gene and the centromere. With independent assortment of the eight chromatids, on the other hand, a 9:9:9:24:24:24:24:64 ratio would be expected, For the small population used here, it is not possible to distinguish between these two extreme ratios by means of the χ^2 test. In a study of this sort, however, the most critical evidence comes from the pattern of incompatible group reactions rather than from the close fit of obtained frequencies of different genotypes to expected ratios.

TABLE 1

AVERAGE NUMBER OF SEEDS SET PER HEAD (10 FLOWERS) IN ALL POSSIBLE SELF-POLLINATIONS, DIALLEL CROSSES AND RECIPROCAL BACK-CROSSES AMONG 29 F_1 PLANTS AND THEIR PARENTS OF 64-CHROMOSOME WHITE CLOVER. ALL CROSSES OR SELFS INCLUDED WITHIN THE BOXES FORMED BY THE HEAVY LINES ARE CONSIDERED INCOMPATIBLE. THE DESIGNATIONS II, III AND IV FOR THE DIFFERENT GENOTYPES REPRESENT THE PROPOSED DIALLELIC, TRIALLELIC AND TETRAALLELIC CLASSES

♀ GENOTYPE	NO. PLANTS	AVERAGE[a] SEEDS PER HEAD: CROSSES WITH FOLLOWING ♂ GENOTYPES: II-♀ P	II-♂ P	II-1	II-2	III-1	III-2	III-3	IV	SELF-POLLINATIONS: GREENHOUSE: 10 FLS. PER HEAD	GREENHOUSE: ENTIRE HEADS MANIPULATED	FIELD	LEAST SIGNIF. DIFF. BETWEEN COMP. CROSSES AND SELFS
II-♀ P	1	...	26.7	16.9	12.8	28.7	26.8	22.7	20.6	0.0	0.1	...	5.5
II-♂ P	1	21.0	...	20.0	16.5	23.5	22.4	21.7	23.4	0.0	0.1	...	8.5
II-1	1	7.7	0.0	...	19.5	18.5	18.3	17.7	18.2	0.0	0.7	...	6.9
II-2	2	0.1	5.7	4.3	0.0	11.0	21.0	12.8	13.6	0.2	0.0	1.8	3.6
III-1	4	3.0	1.7	1.8	1.5	12.5	19.2	11.4	13.0	17.7	96.2	32.0	3.2
III-2	4	2.2	13.0	6.0	15.5	16.1	20.5	15.7	16.1	25.3	81.0	25.8	5.3
III-3	3	0.5	4.5	0.7	1.7	13.9	22.2	10.7	13.8	21.1[b]	110.7	31.4	5.5
IV	15	1.0	1.2	1.4	1.5	11.2	15.8	11.2	10.9	15.6	73.3	15.0	2.1

[a] All averages for the II genotypes used as ♀ were weighted according to the number of crosses involved, and consequently the least significant differences were computed according to the average number of crosses within the different male genotypes. On the other hand, the averages for the III and IV genotypes used as ♀ were obtained from the individual plant averages within each class, and consequently each average was based on the same number of variates for computing the least significant differences.

[b] Since this average was based on only two plants, the data for selfing were not included in the analysis for the ♀ genotype III-3.

In order to test the behavior of oppositional alleles in this doubled condition, each of the 29 F_1 plants from the 64 × 64 cross was self-pollinated, crossed reciprocally to both parents, and crossed diallely with all of the sister F_1 plants. The results (table 1) were more complicated than those from the 32 × 32 cross, and they cannot be explained by any of the existing hypotheses. Although the 64-chromosome parents were self-incompatible like their 32-chromosome counterparts, the F_1 generation segregated into three self-incompatible plants and 26 self-compatibles. These 29 plants were self-pollinated by tripping 10 flowers per head in the greenhouse and by manipulating entire heads in both greenhouse and field. The classification into compatible and incompatible types was very distinct by all three methods, and the results from the three methods agreed (table 1). The differences in average seed set between the 26 self-compatible plants were highly significant in each of the three methods, but there were no significant correlations between methods. The value of r was 0.36 when correlating the two greenhouse tests, and it was only 0.10 when correlating the results of manipulation in greenhouse and field.

In order to explain the 3 : 26 segregation, it is proposed that diallelic plants, which produce only one kind of heterozygous pollen, are self-incompatible, whereas triallelic and tetraallelic plants, which produce, respectively, three and six kinds of heterozygous pollen, are self-compatible. It must be assumed: (*a*) When only one type of heterozygous pollen is produced, the presence of one or both of the same factors in a pistil will inhibit that pollen, and (*b*) when more than one type of heterozygous pollen is produced by a plant, none of it is inhibited even if the same factors are present in the pistil. [Assumption (*a*) has been verified by the work of Lewis,[7] but assumption (*b*) is new to this subject; no previous studies have included comparable diallelic, triallelic and tetraallelic genotypes in all possible combinations.] On the other hand, homozygous pollen is assumed to be inhibited whenever the same allele is present in the pistil. Such a proposal is more complex than would be needed to explain only the differences in self-compatibility, but in this form it is also useful in explaining certain cross-incompatibilities. This concept implies that pollen from a tetraploid is not always specifically inhibited by the same genes in the pistil, as is the case with diploids; the ability or inability to grow depends instead on the reaction between the stigma and all the types of pollen placed upon it. Perhaps chromosome doubling has brought into action certain modifying genes that did not express themselves in the diploid, similar to the results obtained by Randolph[8] and Fischer.[9] In crossing the two balanced diallelic parents, which were shown by their 32-chromosome sister cuttings to be different in all four alleles, the F_1 should consist of a 1:8 ratio of self-incompatibles:self-compatibles (i.e., four diallelic:32 triallelic or tetraallelic). The obtained ratio 3:26 fits the expected 1:8

TABLE 2

THEORETICAL COMPATIBILITY REACTIONS FOR THE DIALLEL INTERCROSSES AND RECIPROCAL BACKCROSSES WITH AN F_1 POPULATION OBTAINED BY CROSSING TWO DIALLELIC 4*n* PARENTS WHICH DIFFERED IN ALL FOUR ALLELES. A + SIGN INDICATES THAT THE POLLEN SHOULD FUNCTION, AND 0 THAT IT SHOULD NOT. THE REACTION WITH THE HOMOZYGOUS TYPES IS SHOWN ON THE UPPER LINE IN EACH BOX AND THE HETEROZYGOUS IN THE LOWER. SEE TEXT FOR HYPOTHESIS EXPLAINING GROWTH OF HOMOZYGOUS AND HETEROZYGOUS POLLEN. ALL CROSSES INCLUDED WITHIN THE BOXES FORMED BY THE HEAVY LINES ARE CONSIDERED INCOMPATIBLE

		POLLEN TUBE REACTIONS OF GENOTYPES PRODUCING THE FOLLOWING GAMETES:										
		A	B	C	D	E	F	G	H	I	J	K
	♀ GENOTYPES WITH EXPECTED FREQUENCY	1 S_1S_1 1 S_2S_2 4 S_1S_2	1 S_3S_3 1 S_4S_4 4 S_3S_4	1 S_1S_1 1 S_3S_3 4 S_1S_3	1 S_1S_1 1 S_4S_4 4 S_1S_4	1 S_2S_2 1 S_3S_3 4 S_2S_3	1 S_2S_2 1 S_4S_4 4 S_2S_4	1 S_1S_1 1 S_3S_4 2 S_1S_3 2 S_1S_4	1 S_2S_2 1 S_3S_4 2 S_2S_3 2 S_2S_4	1 S_3S_3 1 S_1S_3 2 S_1S_3 2 S_1S_4 2 S_2S_3	1 S_4S_4 1 S_1S_3 2 S_1S_4 2 S_2S_3 2 S_2S_4	1 S_1S_3 1 S_1S_4 1 S_2S_3 1 S_2S_4 1 S_3S_4
A	♀ Parent	0 0	+ +	0 +	0 +	0 +	0 +	0	0	+	+	
	$S_1S_1S_2S_2$	0	+	0	0	0	0	+ + +	+ + +	+ + +	+ + +	+ + +
												+ + +
B	♂ Parent	+ +	0 0	+ 0	+ 0	+ 0	+ 0	+	+	0	0	
	$S_3S_3S_4S_4$	+	0	0	0	0	0	+ + +	+ + +	+ + +	+ + +	+ + +
												+ + +
C	1 $S_1S_1S_3S_4$	0 +	0 +	0 0	0 +	+ 0	+ +	0	+	0	+	
		0	0	0	0	0	+	+ + +	+ + +	+ + +	+ + +	+ + +
												+ + +

D	$1\ S_1S_1S_4S_4$	0 + 0	+ 0 0	0 + 0	0 0 0	+ + +	+ 0 0	0 + + +	+ + + +	+ + + +	0 + + +	+ + + + + +
E	$1\ S_2S_2S_4S_4$	+ 0 0	0 + 0	+ 0 0	+ + +	0 0 0	0 + 0	+ + + +	0 + + +	0 + + +	+ + + +	+ + + + + +
F	$1\ S_2S_2S_4S_4$	+ 0 0	+ 0 0	+ + +	+ 0 0	0 + 0	0 0 0	+ + + +	0 + + +	+ + + +	0 + + +	+ + + + + +
G	$4\ S_1S_1S_3S_4$	0 + 0	0 0 0	0 0 0	0 0 0	+ 0 0	+ 0 0	0 + + +	+ + + +	0 + + +	0 + + +	+ + + + + +
H	$4\ S_2S_2S_3S_4$	+ 0 0	0 0 0	+ 0 0	+ 0 0	0 0 0	0 0 0	+ + + +	0 + + +	0 + + +	0 + + +	+ + + + + +
I	$4\ S_1S_2S_3S_3$	0 0 0	0 + 0	0 0 0	0 + 0	0 0 0	0 + 0	0 + + +	0 + + +	0 + + +	+ + + +	+ + + + + +
J	$4\ S_1S_2S_4S_4$	0 0 0	+ 0 0	0 + 0	0 0 0	0 + 0	0 0 0	0 + + +	0 + + +	+ + + +	0 + + +	+ + + + + +
K	$16\ S_1S_2S_3S_4$	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 0 0	0 + + +	0 + + +	0 + + +	0 + + +	+ + + + + +

very closely with a non-significant corrected $\chi^2 = 0.031$. Similarly, it fits the expected 9:40 ratio, based on random chromatid assortment, with a non-significant corrected $\chi^2 = 0.767$.

When the three self-incompatible F_1 plants were diallely inter-crossed and reciprocally back-crossed to both parents, their differential reaction allowed them to be divided into two groups (table 1). One of the three plants (Group II-1) was compatible as female with its female parent but incompatible with its male, while the other two (Group II-2) were incompatible as females with their female parent, compatible with their male, and cross-incompatible. Although the crosses II-1 × II-♀ P, II-2 × II-♂ P and II-2 × II-1 are considered compatible, it should be pointed out that in every case the average seed set was significantly lower than that obtained in the other compatible crosses of these same three plants as females with their 26 self-compatible sisters.

All crosses involving the self-compatible plants as males were compatible, but several differential reactions occurred among the crosses self-compatible ♀ × self-incompatible ♂ (table 1). Four self-compatible plants (Group III-1) set significantly more seed when crossed in this direction with their female parent than they did with the other self-incompatible plants. This average of 3.0 with the female parent, however, showed a highly significant difference in comparison with the compatible crosses between the same four plants as females and their self-compatible sisters as males. Four other self-compatible plants (Group III-2) proved incompatible with their female parent when the latter was used as male but compatible with the other three self-incompatible groups used as males. In this case, the cross III-2 × II-1 has been classed as compatible, but its average of 6.0 was significantly different from all other compatible crosses using Group III-2 as female. A third group of three self-compatible plants (Group III-3) set an average of 4.5 seeds per head when crossed as females with their male parent, and the differences between this and the crosses with the other self-incompatible groups as males were highly significant. Likewise, the differences between 4.5 and the other compatible crosses using Group III-3 as female were highly significant. The remaining 15 self-compatible plants (Group IV) proved incompatible as females with both parents and with both self-incompatible F_1 groups. The differences between the four types of incompatible crosses involving Group IV as female were not significant.

If pollen-tube growth in crosses is conditioned in the same manner as was proposed for selfings, the theoretical pattern of incompatibilities (table 2) is similar to that which was obtained (table 1). The segregation of 1:2:4:4:3:15 in the F_1 population gives a very satisfactory fit to expected, with a non-significant $\chi^2 = 4.904$ (7 D.F.). When the same ratio was fitted to the expected based on independent chromatid assortment, a non-

significant $\chi^2 = 7.805$ was obtained. Probably because the F_1 population was small, only two self-incompatible groups were found. In order to fit these results to the pattern of cross-incompatibilities obtained with the self-compatible plants as females, it is apparent that the two self-incompatible groups which were obtained were either C and E or D and F (table 2). The data do not fit in all respects, however, and certain additional assumptions must be made. One discrepancy from the expected results was that those self-compatible plants (Group III-1) which were compatible as females with their female parent, but incompatible with their male parent should have been incompatible as females with one or the other of the self-incompatible F_1 groups but not with both. Each of the four plants in this group gave some indication of setting more seed with one self-incompatible F_1 group than with the other, but the differences were neither large enough nor consistent enough to be significant. The second and more serious deviation from expected was the incompatibility obtained between each of the self-incompatible F_1 plants as female and one of their parents. According to the hypothesis, these crosses should have been compatible, and no adequate explanation of this irregular behavior is available at present.

Two additional lines of evidence have been obtained which support the concept of pollen-tube growth sometimes depending on the interaction between the stigma and all pollen grains placed upon it rather than on the specific relation between the alleles borne by the pollen and the stigma. In the first place, significant differences in average seed set were obtained from the different male groups. No such effect was observed in the 32×32 chromosome test, nor has it ever been observed before in white clover in the thousands of crosses which have been made with normal 32-chromosome plants. Several cases of morphological or cytological abnormalities causing defective pollen and consequent male sterility have been observed in 32-chromosome clover, but no differences due to genetic causes, where there was no influence on pollen fertility, have been demonstrated. Among the 64×64 matings, some of the most outstanding cases have already been pointed out. Further examples may be seen (table 1) by comparing the differences between group averages with the least significant differences. Similar differences were found when the results were grouped in other ways. For example the average of all crosses using the triallelic plants as males was 15.4, while the average for the tetraallelic as males was 13.4, and the difference between these averages was highly significant. Similarly, the triallelic plants set significantly more selfed seed (av. = 21.4) than the tetraallelic plants (av. = 15.6). One possible explanation of these relationships might be that more complex interactions and somewhat lowered seed set would result from six types of heterozygous pollen on the stigma than from three; further tests are now being made. With these data it

was possible to demonstrate in addition highly significant differences between the plants within each of the groups in regard to their differential male influence. It was also noticed that with most of the self-compatible plants, the seed set from selfing was significantly greater than from crossing. Most of the selfs were made without emasculation, whereas the crosses were made following emasculation, but differential treatment of this sort has never given different results with normal 32-chromosome plants. It is necessary to postulate modifying factors to explain such behavior, and there may be a better chance for their expression in tetraploid plants.

The other additional line of evidence supporting the interaction hypothesis was derived from the significant end-season increases in seed set obtained in the green house. This behavior likewise has not been observed in 32-chromosome white clover. When it was noticed that the reflection of pedicels was not so definite a reaction with the 64-chromosome plants as it was with the 32, 103 crosses were made in duplicate. The first crosses of each pair averaged 13.8 seeds per head, while the second averaged 17.2. The average interval between them was 10.9 days. The differences between repeats were analyzed for each of the eight types of compatible matings between diallelic, triallelic and tetraallelic plants, but significant variance due to repeats was found only for the crosses IV $\times$ III and IV $\times$ IV. These combinations would involve the greatest number of interactions between pollen and pistil, and therefore might show the greatest influence from environmental factors such as end-of-season.

Summary.—The 64-chromosome stolons of white clover, induced by colchicine treatment of seedlings, were found to be self-incompatible like the 32-chromosome plants from which they arose. In the F_1 population from two of the 32-chromosome plants, four intra-sterile, inter-fertile groups were found, and all F_1 plants were reciprocally compatible with both parents, indicating that the parents differed in all four oppositional alleles. In the F_1 from the two corresponding 64-chromosome stolons, three plants were self-incompatible, and the remaining 26 were self-compatible. All intercrosses involving the self-compatible plants as males were compatible, but both compatible and incompatible matings were found when the self-incompatible parents and F_1 plants were used as males. On the basis of these differential reactions, several genotypes were postulated. It is suggested that growth of pollen bearing two different alleles depends sometimes on the interaction between the stigma and all pollen placed upon it rather than on a specific oppositional effect.

[1] Contribution No. 58, of the U. S. Regional Pasture Research Laboratory, Division of Forage Crops and Diseases, Bureau of Plant Industry, Soils, and Agricultural Engineering, Agricultural Research Administration, U. S. Department of Agriculture, State College, Pennsylvania, in coöperation with the northeastern states.

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LINKAGE STUDIES OF THE RAT (RATTUS NORVEGICUS) VI.

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Communicated February 28, 1944

1. *A Fourth Linkage Group.*—In a canvass of the linkage relations of genes of the rat, it has been found that *kinky* and *stub* are borne in a common chromosome. The evidence is as follows. Kinky (*k*) is a recessive mutant gene discovered by Feldman.[1] It makes the hair short and curly, being similar in its phenotypic effects to the two dominant mutations curly and curly$_2$. Stub (*st*) is a mutation likewise recessive and frequently lethal at an early age, discovered by King.[2] It makes the tail short and "stubby" and frequently results in abnormality of the kidneys, and other organs in the pelvic region, the hind legs being sometimes partially fused. Microphthalmia also is often observed, particularly of the left side. Since stub animals, even when they live to normal breeding age, are usually sterile, only animals heterozygous for the stub gene are available for genetic study. When they are crossed with animals homozygous for kinky, half the resulting progeny will be heterozygous for both genes, though they show neither. Such carriers can be identified by suitable test matings. They should theoretically produce gametes of four classes, *k*, *st*, *k st* and *O*. The first two would be non-crossover (repulsion) gametes, the last two would be crossover (recombination) classes. To ascertain the relative frequency of their occurrence is a task best undertaken by an outcross of an F_1 animal to one which carries neither gene. The resulting progeny may at maturity be tested individually by a backcross to an F_1 animal known to be a carrier of both *k* and *st*. Usually it is most convenient to employ out-crossed females in back-crossing to tested F_1 males. A total of 138 out-crossed females have thus been tested. They fall into the four expected classes with frequencies as indicated, $\frac{k}{66}\ \frac{st}{25}\ \frac{k\,st}{11}\ \frac{O}{36}$; crossovers $11 + 36 = 47$; non-crossovers $66 + 25 = 91$. Total number 138. Deviation from equality

of the two groups 22, P.E. 3.96. Dev./P.E. = 5.5, a significant and clear demonstration of repulsion between the two genes. Crossover percentage 34.1 ± 2.6.

It will be observed that of the 138 tested animals 77 are recorded as carriers of the *k* gene. This is a little more than the expected half. But only 36 tested animals are recorded as carriers of *st*, which indicates that many such failed of identification in the test matings through the production of too small a number of young (six or more being rated a satisfactory test) or because the stub young died at or before birth and were eaten before the test litters were inspected.

Consequently it would seem desirable to correct the deficiency of stub carriers by increasing the number to half the total population, and decreasing correspondingly the classes recorded as lacking *st*. The deficiency of stub carriers is 33, requiring an increase of 16 each in classes *k st* and *st*, and a corresponding decrease in classes *O* and *k*. Making these changes the classes would become $\frac{k}{50}\ \frac{st\,k\,st}{41\ \ 27}\ \frac{O}{20}$, a more symmetrical distribution, though with an unchanged crossover percentage, 34.1.

Before indications of linkage between stub and kinky had been obtained, tests had been made with entirely negative results for linkage of stub with wobbly (*wo*), with blue dilution (*d*), and with Curly$_2$ (*Cu*$_2$).

2. *A Further Study of the Second Linkage Group.*—In earlier studies[3] it has been shown that the dominant gene curly is loosely linked with the recessive and lethal gene anemia (*an*) and the recessive gene incisorless (*in* complete absence of incisor teeth).

To get a better knowledge of the order of the genes and their map distances in the curly region, a three-point cross has been made between curly animals (carrying anemia) and incisorless animals. The resulting F_1 animals were in genetic formula $\frac{Cu\ an\ +}{+\ +\ in}$. They were out-crossed to animals free from all three mutant genes, and the out-crossed progeny were then back-crossed to F_1 to ascertain how many and which of the three genes each of them carried. A total of 175 animals have been thus tested, and they fall into 8 classes. Non-crossover classes are represented by the genotypes *Cu an*, 65 individuals, and *in*, 76 individuals. The six crossover genotypes and their frequencies are as follows:

Cu in	7	*An in*	3
an	3	*Cu an in*	5
Cu	5	*O*	11, total 34.

It was shown in an earlier publication that *an* probably lies between *Cu* and *b* (brown) though much closer to the former. If the order of the three

genes is as assumed *Cu an b*, the position of *in* with reference to *Cu an* may be either (1) *Cu an in* or (2) *in Cu an* or (3) *Cu in an*. Let us consider the evidence for each of these possible arrangements.

Whatever the order of the three genes, the total occurrences of crossing over (single or double) in the 175 cases studied will be the same, 34. But the number of double crossovers will be different under each assumed arrangement. Thus it will be 8 under order (1), 10 under order (2) and 16 under order (3).

ORDER	SINGLE	DOUBLE	% DOUBLE
(1) *Cu an in*	10 + 16 = 26	8	23.5
(2) *in Cu an*	16 + 8 = 24	10	29.4
(3) *Cu in an*	10 + 8 = 18	16	47.0

That order is most probable under which double crossovers are least absolutely and relatively. This points to order (1) as most probable and order (3) as least probable. Order (3) is further improbable because it would make the crossover percentage between *Cu* and *an* 28.5, whereas an earlier study in which *in* was not involved (and in which consequently the apparent crossing over between *Cu* and *an* would be less) showed only 2.1 ± 0.5%. Under either order (1) or order (2) the value for *Cu an* would in this investigation be 10.3%, a much smaller discrepancy.

Assuming that order (1) is correct, the total amount of crossing over in the region *Cu an* will be 10 single + 8 double = 18 = 10.3 ± 1.6%. In the region *an in* there will be 16 single + 8 double = 24 = 13.7 ± 1.7%. Consequently the map for the second chromosome may be drawn thus:

	Cu	*an*	*in*	*b*
II.	0	10.3	24	45

A test of the validity of this arrangement by a study of the linkage relation of *in* to *b* is highly desirable.

For comparison we may add here the other linkage maps of the rat so far as at present known.

I.	*l*	*c*	*r*	*p*	*w*
	0	3.3	3.8	23.3	58.6

III.	*hr*	*wo*
	0	40.3

IV.	*k*	*st*
	0	34.1

[1] *Jour. Hered.*, **26**, 162 (1935).
[2] *The American Anatomical Memoirs*, No. 17, p. 59 (1939); *Anat. Rec.* **81**, p. 283 (1941).
[3] *Proc. Nat. Acad. Sci.*, **21**, 390 (1935); Ibid., **26**, 578 (1940). *Carnegie Institution Year Book*, No. 41, p. 225 (1941–1942).

EQUILIBRIUM IN GENIC MATERIALS

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Communicated February 25, 1944

Inbred strains of maize, continuously inbred for many generations, are favorable material to show small heritable variations. Changes in cob and silk color, plant and ear size, and in the amount of chlorophyll have been reported (Jones, 1924, 1939; Singleton, 1943). These alterations either have no effect upon vigor and productiveness or clearly reduce the plants in some degree. In addition to these, other heritable changes have been found recently in various inbred material. All of these lessen the ability of the plant to grow and to reproduce itself in some measure. In their total effect upon the inbred plants, they appear to be degenerative changes.

Unexpectedly, it has been found that these apparently degenerate lines, when crossed back with their parental lines, show heterosis. Presumably, they differ by only a single gene. This point remains to be tested. Furthermore, one of these degenerate lines has been shown to give better results, than the parental normal line, in outcrosses. The changes so far noted affect both physiological and morphological characters and are listed as follows:

INBRED LINE	CHARACTERISTIC FEATURE	NATURE OF CHANGE
C14[a]	Late flowering	Tassels and silks appear later
C14	Blotched leaf	Chlorophyll reduced
C30	Reduced plant	Smaller in all parts, blooms earlier
C237	Reduced ear	Ears smaller, stalks taller
Illinois Hy	Dwarf plant	Internodes reduced in length
C.I. 4–8	Narrow leaf	Leaf width and stalk diameter smaller, blooms earlier
C.I. Kr (187-2)	Crooked stalk	Internodes shortened and bent
C.I. Kr (187-2)	Pale top	Chlorophyll reduced

[a] The letter C designates inbreds produced at the Connecticut Agricultural Experiment Station, New Haven, Connecticut. Illinois refers to the Agricultural Experiment Station at Urbana, Illinois, and C.I., the Office of Cereal Investigations, U. S. Department of Agriculture, Washington, D. C.

The reduced plant, C30, has been described by Singleton (1943, 1944). It is a change in the Purdue 39 inbred sweet corn originally found in Idaho. It is used in the production of Connecticut hybrid sweet corn. This single gene mutation gives evidence for increased growth in crosses with unrelated lines as well as a hybrid vigor effect when crossed with the parental line.

Increased growth in crosses of related lines was noted during the 1943 growing season in the narrow leaf line crossed back to the original plants from which it was derived. The narrow leaf condition was found two years previously in one progeny that had been grown from a single self-fertilized ear. When grown the following year in adjoining rows it was seen to be clearly distinct in width of leaf. It was crossed back to the original line to see if it would segregate as a single factor difference. When the original line, the deviating line and their first generation hybrid were all grown in adjoining rows in 1943, differences were clearly noticed.

The originally 4-8 line and the same line from a new source were alike, showing that no visible change had occurred in the parental line. The deviating narrow leaf line was smaller in leaf width and stalk diameter, the same or slightly taller in height of stalk, the same in time of shedding pollen but earlier by seven days in time of silking. Thus it was not a degeneration in all respects as earliness of flowering is a good measure of growth efficiency in maize. Any environmental or inherited condition that favors growth is reflected in a speeding up of the time of flowering.

The first generation hybrid plants resulting from the cross of the deviating narrow leaf line and the original line from which it came was equal to or better than the better parent in all characters. The combined result was a noticeably greener, and more robust plant that was early maturing and more productive of grain. Not enough plants were grown to measure these small differences accurately. The hybrid did not surpass either parent in any single character by a statistically significant margin but the total result was impressive, especially in yield of grain.

Illinois Hy is an inbred that is widely used in the production of hybrid field corn. A dwarf form of this inbred was compared with the original line for two years. It has proved to be typical Hy in all details of color and structure except that the internodes are shorter, and the leaves smaller. The plant is brachytic in type, averaging about half the height of the original line. The dwarf plants flower at the same time as their normal parent and produce smaller ears with smaller kernels and are somewhat less productive in total amount of grain. While the segregation of this character has not been tested, dwarfs of this type have appeared in other lines and are usually due to a single mendelizing recessive gene.

The first generation of the cross of dwarf and normal Hy was grown alongside the normal plants. The crossed plants averaged the same in height but shed pollen two days earlier and silked four days earlier. The

plants were noticeably greener and more thrifty in appearance during the growing season and at maturity the ears were larger with brighter and better filled kernels. As the hybrid vigor effect was not anticipated only a few plants were grown and most of the ears were used for hand pollination so that no yield figures are available. The earlier flowering and maturing is a clear indication of heightened growth efficiency.

An inbred line of Leaming, C14, has been continuously self-fertilized for 21 generations. After the first ten generations it was reduced to a high degree of uniformity and constancy. In the 17th generation a conspicuously tall darker green plant, flowering early, was noted in one progeny. This single plant had the appearance of a typical C14 slightly enlarged and invigorated as if it was growing in a particularly fertile spot. It was not large enough to be an outcross such as appear occasionally as a result of contamination with pollen from unrelated plants. This deviating plant was self-fertilized and the resulting ear was larger and better matured than the other self-fertilized ears in the same progeny. The kernels were marked with a faint pericarp stripe not characteristic of any other varieties that had been grown that year or before.

The progeny grown from this ear was noticeably taller, greener and more productive than the other related lines. It was slightly more variable but showed no clear segregation. Several plants were self-fertilized and grown for two successive generations. Some of these now differ from the original line in glume and internode color and in the amount of chlorophyll blotching shown by the leaves late in the season. All progenies have been reduced to the same level of productiveness as the original line or below.

The segregation of color markings indicates that the original plant could have resulted from an outcross with a related back-crossed line that was growing nearby the year before the deviating plant was discovered. It was so considered until the behavior of the other deviating lines was noted. The possibility that this is a mutation originating in the heterozygous condition must now be considered.

One line from this source more heavily blotched than the original is now noticeably less vigorous and productive. Whatever its origin it differs from the original C14 line by only a small germinal alteration. When crossed with the normal line the first generation hybrid plants were taller, greener and more productive. Here again the differences are small and not statistically significant for any one measurable character but all are in the same direction of increased growth efficiency.

The reduced ear size character has been described previously (Jones, 1939) as a change in two directions. The number of kernels on the ear is reduced but the height of plant is increased. Crossed with the original this deviating line showed no increasing effect in either height of stalk or

production of grain. The first generation plants were intermediate in both respects.

A single factor mutation in tobacco (Jones, 1921) gives results in the heterozygous condition that are superior in some respects to either parent. In this case the change is from the determinate to the indeterminate habit of growth. The heterozygous plants average several more leaves and more flowers per plant than the determinate parent and produce a larger amount of total plant material than either parent in the same period of growth.

Several other deviating lines in maize have been found but have not yet been compared in crosses with their parental lines. Late flowering plants appeared in another progeny of C14 also in the 17th generation. The seedlings germinate more slowly and the plants are shorter than their normal sibs throughout the growing period. At the end of the season, when the normal plants cease to increase in height, the late flowering plants continue growth for a longer time until they are either as tall or almost as tall as normal when elongation ceases.

In 1942 the normal line averaged 84.0 inches in height to the tip of the tassel and the late flowering line 78.9. In 1943 the heights are 78.2 and 75.9, respectively. In time of flowering in 1943 the two lines compare as follows:

DAYS FROM PLANTING TO	FIRST TASSEL	LAST TASSEL	FIRST SILK	LAST SILK
Late flowering line	63	67	66	78
Normal flowering line	59	65	61	66
Differences	4	2	5	12

The number of days is counted from date of planting to pollen shedding and first appearance of silks. The results are based on an average of four replicated plots in each case. The two lines differ in no morphological character and at the end of the season appear to be alike in every respect.

In 1942 several progenies of C.I. Kr (187-2) were grown from single self-fertilized ears all coming from a single line that had been inbred for many years and grown in Connecticut four generations with continuous self-fertilization. No unusual variations had been noted in previous years. In 1942 several progenies were segregating for an obscure chlorophyll variation. A few plants were noted in the early stages of growth as being shorter and lighter in color at the base of the upper leaves. These plants were marked and self-fertilized. At time of flowering the differences had largely disappeared, the plants were entirely normal in appearance but were usually a few days later in silking. At maturity the plants were so nearly the same as the normal plants in height and productiveness that they could not be noted as deviations.

When grown the following year two progenies were homozygous for this

pale top condition, four were segregating and six were free of it. In the homozygous progenies the plants were now noticeably reduced in size throughout the season. The stalks were more slender and shorter at maturity. One homozygous pale top progeny measured 72.8 inches in height compared to six normal progenies that averaged 76.8. The plants flowered later and the ears were smaller and less well matured. Chlorophyll color was a little lighter throughout the season although the plants grew out of the early pale top condition and were not clearly differentiated in this respect after flowering. Only when they were growing together in an entire row were they distinctly different. Nevertheless, the deviating plants appeared to be more differentiated from normal the second year than when they first appeared as segregates from normal. The condition had become progressively worse.

In one of the progenies, segregating for pale top in 1942, one normal green plant was noted as distinctly shorter at maturity. Upon closer examination it was seen that two internodes at the base of the plant were much shortened and bent. The progeny grown from the self-fertilized seed of this plant were all crooked in the same way as the parent, varying in the number of internodes affected from one to five. In some plants alternate internodes are shortened and bent, the intervening internodes being normal.

Out of thirteen progenies grown, six are normal, four are segregating normal and crooked stalk, and three are all crooked. In two of the latter the parental plant was not noted as being abnormal in stalk formation. All of the progenies showing crooked stalks are also either homozygous or heterozygous for pale top and all of the progenies showing pale top are either homozygous or heterozygous for crooked stalk. Apparently the two deviating types originated at the same time. They both behave as mendelian recessives. One progeny is also segregating for flat cobs. Further tests are needed to show whether or not these three conditions are associated in any way and the meaning of the simultaneous appearance of the three degenerate types.

Neither pale top nor crooked stalk have been tested in crosses with normal related or unrelated lines. But there is an indication that the normal plants heterozygous for one or both conditions are taller than homozygous normals. Two progenies segregating for pale top and crooked stalks averaged 80.1 inches in height compared to the six normal progenies which averaged 76.8 in adjacent rows. The segregating progenies had several deviating plants that were clearly shorter than normal and these were included in the measurements. The heterozygous normal plants must be taller than the homozygous normal to more than make up for this difference.

In all cases so far observed the deviating lines originate in heterozygous

normal plants that are selected either wittingly or unwittingly for propagation. The larger growth of the heterozygous plants increases the chance that they will be used as the progenitors for the succeeding generations. The maintenance of inbred strains to be used in the production of hybrid corn is a more difficult problem than was at first realized. Even if the reduced lines do give better results in crosses the propagation of the inbred line may become prohibitively costly. This apparently has happened in several widely used inbreds which are now so unproductive as to be difficult to produce in those regions where the hybrids from them are still among the most productive.

The increased growth shown by some and possibly all of these related crosses results in each case from a single allelic difference. This at first sight appears to be a stimulus of heterozygosis as originally postulated by East (1909), East and Hayes (1912) and Shull (1914). This conception was extended by Rasmusson (1934) and by East (1936) as an interaction between alleles. East dismissed these defective genes as having no importance in heterosis. It is now apparent that no clear distinction can be made between normal and degenerate characters since they may have multiple effects, some favorable, some unfavorable. Dunn and Caspari (1942) describe numerous changes in a small section of a mouse chromosome having diverse effects, some lethal, others having degenerate effects. Hybrid combinations of some of these multiple changes promote normal growth.

Deviating lines show a loss in some respects and a gain in others. The earlier flowering of the narrow leaf and reduced plant lines is an indication of increased growth efficiency. There seems to be an equilibrium in genic substances such that a change in one direction may be counter-balanced by a change in another. Whatever the deviating line has lost the original line supplies in their crossbred offspring. What the deviating line has acquired may be added to the sum total of genic action. Along with this there is the accumulation of non-allelic, favorable growth factors that is known to occur.

This investigation has been aided by a grant from the Rockefeller Foundation.

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ON THE RÔLE OF CARBON DIOXIDE IN THE METABOLISM OF CLOSTRIDIUM THERMOACETICUM

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Communicated March 2, 1944

Clostridium thermoaceticum ferments glucose anaerobically with the formation of somewhat more than 2.5 moles of acetic acid per mole of glucose decomposed.[3] Besides acetic acid, only cell material and a trace of carbon dioxide are produced. The remarkable features of this "acetic acid fermentation" are (1) the formation of more than two moles of acetic acid per mole of glucose and (2) the absence of carbon dioxide or other C_1 compound which would be expected to arise by a C_1-C_2 split of a C_3 intermediate.

In explanation of the high yield of acetic acid and the absence of carbon dioxide, two hypotheses have been suggested.[3] One is the primary cleavage of glucose into three C_2 fragments. The other is the production and later reabsorption of carbon dioxide. The latter hypothesis seems much more probable in view of the fact that two other anaerobic spore-formers are already known to convert carbon dioxide into acetic acid. The rôle of carbon dioxide in the metabolism of these bacteria is that of an oxidizing agent. *Clostridium aceticum*[4, 5] uses it for the oxidation of molecular hydrogen according to the equation

$$4H_2 + 2CO_2 = CH_3COOH + 2H_2O$$

while *Clostridium acidi-urici*[1, 2] uses it for the oxidation of uric acid and other purines. The acetic acid fermentation of *C. thermoaceticum* may similarly be regarded as an anaerobic oxidation of glucose by means of carbon dioxide as expressed by the equations

Oxidation:	$C_6H_{12}O_6 + 2H_2O$	$= 2CH_3COOH + 2CO_2 + 8H$
Reduction:	$8H + 2CO_2$	$= CH_3COOH + 2H_2O$
Net reaction:	$C_6H_{12}O_6$	$= 3CH_3COOH$

There are two direct methods by which the above hypothesis regarding the rôle of carbon dioxide in the acetic acid fermentation might be tested. One involves the use of carbon dioxide labeled with a carbon isotope. This method cannot at present be applied because of wartime restrictions and the recent untimely death of Dr. S. Ruben who was planning to cooperate in these experiments. The second method consists of studying the action of the bacteria on a substrate more reduced than glucose, such as glycerol or a sugar alcohol. Since glucose is fermented without carbon dioxide evolution, it follows that the decomposition of a more reduced sub-

strate must involve carbon dioxide uptake if carbon dioxide is acting as an oxidizing agent. Unfortunately this method also cannot be applied to the problem at hand because the strain of *C. thermoaceticum* at present available does not attack any of the polyalcohols.

Since no direct method of detecting carbon dioxide utilization is available, an indirect approach based upon the following line of reasoning has been used. If glucose decomposition involves a splitting of the molecule into three C_2 pieces (the first hypothesis mentioned above) the decomposition of a pentose should yield two C_2 and one C_1 fragments. If, on the contrary, acetic acid is synthesized entirely or in part from carbon dioxide, it should be the only product of a pentose as well as a hexose fermentation. The experimental results (table 1) show that except for traces of carbon dioxide, acetic acid is the only product formed from *l*-xylose. Therefore a utilization of carbon dioxide is indicated.

TABLE 1

FERMENTATIONS OF *l*-XYLOSE AND PYRUVATE BY *Clostridium thermoaceticum*

(mM per 100 mM substrate fermented)

	SUBSTRATES	
PRODUCTS	*l*-XYLOSE	PYRUVATE
Carbon dioxide	2.3	51.3
Acetic acid	223	109
Carbon recovery (%)	90	89
Redox index	Indet.	1.03

Although the results obtained with xylose definitely favor the hypothesis of carbon dioxide reduction, other explanations are, of course, possible. Instead of being directly split into two C_2 and one C_1 fragments, xylose might be broken into a C_2 and a C_3 piece, two of the latter then being converted into a C_6 compound which could be subsequently split into three C_2 fragments. In this way the facts could be explained without assuming carbon dioxide to be either formed or utilized. In view of this theoretical possibility it seemed desirable to find out if *C. thermoaceticum* is able to form carbon dioxide from an appropriate substrate and to see whether indications of acetic acid synthesis could be simultaneously obtained. The decomposition of pyruvate was therefore studied.

Since pyruvate is more oxidized than a carbohydrate, its decomposition must yield carbon dioxide or some other compound more oxidized than acetic acid. Carbon dioxide and acetic acid are in fact the only products. Now the decomposition of pyruvate would be expected, in the absence of carbon dioxide reduction and the synthesis of C_6 compounds, to yield not more than 100 mM of acetic acid per 100 mM of pyruvate decomposed and not less than 100 mM of carbon dioxide per 100 mM of acetic acid formed. Actually the experimental results (table 1) agree rather well with the equation

$$4CH_3COCOOH + 2H_2O = 5CH_3COOH + 2CO_2.$$

The yield of acetic acid is significantly above 100 mM while the yield of carbon dioxide is only about 50 mM per 100 mM of pyruvate fermented. These results are again entirely consistent with the view that carbon dioxide is converted into acetic acid. But proof of this mechanism must await the application of one of the two direct methods mentioned above.

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THE MATRICES OF FACTOR ANALYSIS

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Communicated February 25, 1944

In factor analysis a study is made of n-rowed symmetric matrices M_0 whose *diagonal elements* are *all zero* and whose nondiagonal elements are correlation coefficients, that is, real numbers between zero and unity. A general problem[1] is then to determine a diagonal matrix D such that

$$M = M_0 + D$$

has minimum rank ρ. The diagonal elements of D are those of the factor matrix M and are called the *communalities*. As soon as D is evaluated a standard computational technique may be employed to write M as a product

$$M = FF',$$

where F has n rows and ρ columns,[2] F' is its transpose.

Professor L. L. Thurstone of the Department of Psychology of the University of Chicago has called my attention to this problem with particular emphasis on a special case. This case is of the greatest importance because the matrices which arise from actual psychological tests appear to be of the type assumed for this case.

Our special problem is the study of the case where ρ is equal to what we shall call the *ideal rank* r. We define r to be the largest order of a nonvanishing minor of M_0 obtained by a selection of r of its rows and r *different* columns. No element of such a minor is a diagonal element of M_0 and

hence all minors of the kind described are minors of M as well as of M_0. It follows that

$$\rho \geqq r,$$

and that our special problem is a study of the case where ρ attains its minimum value r. It should be clear that

$$m = n - 2r \geqq 0.$$

The procedure we shall give then begins with a computation of the ideal rank and will yield not only r but also the corresponding r row and column labels. Then we may alter our notations so that the columns are the *first* r columns and the rows the *second* r rows. This yields a partitioning of M_0 so as to yield the notation

$$M_0 = \begin{pmatrix} A_0 & B' & C' \\ B & C_0 & H' \\ G & H & K_0 \end{pmatrix} \tag{1}$$

for M_0. Here A_0, B and C_0 are r-rowed square matrices and the determinant $|B| \neq 0$. The matrices A_0, C_0, K_0 are symmetric matrices with zero diagonal elements, and K_0, G and H have m rows, G and H have r columns. Let us assume that the computation of r has been carried out so as to yield the matrix

$$E = B^{-1}.$$

We now write

$$M = M_0 + D = \begin{pmatrix} A & B' & G' \\ B & C & H' \\ G & H & K \end{pmatrix},$$

$$A = A_0 + X, \qquad B = B_0 + Y, \qquad K = K_0 + Z \tag{2}$$

for diagonal matrices

$$X = \text{diag.}\{x_1, \ldots, x_r\}, \qquad Y = \text{diag.}\{y_1, \ldots, y_r\},$$

$$Z = \text{diag.}\{z_1, \ldots, z_m\}. \tag{3}$$

Then r is the rank of M if and only if it is the rank of

$$\begin{pmatrix} I & -AE & 0 \\ 0 & I & 0 \\ 0 & -GE & I_m \end{pmatrix} M = \begin{pmatrix} 0 & B' - AEC & G' - AEH \\ B & C & H' \\ 0 & H - GEC & K - GEH' \end{pmatrix}, \tag{4}$$

where I is the r-rowed identity matrix and I_m is the m-rowed identity matrix. This yields the

THEOREM. *The factor matrix M has rank r if and only if X, Y, Z are chosen so that*

$$K = GEH', \quad H = GEC, \quad G' = AEH', \quad B' = AEC. \tag{5}$$

We observe that our hypothesis that r is the ideal rank implies that r is the rank of the $(r + 1)$-rowed square matrix

$$\begin{pmatrix} B & h_j' \\ g_i & k_{ij} \end{pmatrix}$$

obtained by using the i-th row g_i of G, the j-th column h_j' of H', and the element k_{ij} in the i-th row and j-th column of K_0 for $i \neq j$. Then r is the rank of

$$\begin{pmatrix} I & 0 \\ -g_iE & 1 \end{pmatrix}\begin{pmatrix} B & h_j' \\ g_i & k_{ij} \end{pmatrix} = \begin{pmatrix} B & h_j' \\ 0 & k_{ij} - g_iEh_j' \end{pmatrix},$$

that is, $k_{ij} = g_iEh_j'$. But then

$$GEH' - K_0$$

is a diagonal matrix and we satisfy the first relation of (5) by taking $Z = GEH' - K_0$. The second and third relations may be written as

$$GEY = H - GEC_0, \qquad XEH' = G' - A_0EH', \tag{6}$$

and we have the basis for the following:

Determination of Diagonal Elements

I. *Estimate the ideal rank r.* This results in the fixing of our notation for B, A_0, C_0, G, H, K_0.

II. *Find the inverse E of B.* If our estimate of r is too great it will not be possible to carry this out.

III. *Compute* $GE = (\sigma_{ij})$ *where* $i = 1, \ldots, m$ *and* $j = 1, \ldots, r$.

IV. *Compute* $(GE)H'$. The nondiagonal elements of this m by n product will coincide with the corresponding nondiagonal elements of K_0 unless our estimate of r is too small. If they do not coincide the choice of B needs to be modified and it is a simple matter to complete the determination of the inverse of the new B. If they do coincide the diagonal elements of $(GE)H'$ are the desired communalities $z_1, z_2, \ldots, z_m$.

V. *Compute, for every nonzero element* σ_{ij} *of GE, the element in the i-th row and j-th column of* $(GE)C_0$. *Subtract this computed element from the correspondingly placed element of H and divide by* σ_{ij}. The result is y_j, the $(r + j)$-th diagonal element of M. In most cases this will yield m determinations of y_j and a weighted mean obtained by summing elements in columns would probably give a best value.

VI. *Compute* $EH' = (\lambda_{ji})$, *where* $j = 1, \ldots, r$ *and* $i = 1, \ldots, m$. *Find, for every nonzero* λ_{ji}, *the element in the j-th row and i-th column of* $A_0(EH')$

Subtract the result from the correspondingly placed element in G' and divide by λ_{ji}. The result will be x_j, the j-th diagonal element of M.

In the cases most usually encountered the procedure above completely determines all diagonal elements and gives a full solution of our problem. Note that if we determine X so that $G' = AEH'$ as in step VI, then $H - GEC = H - HE'AEC = HE'(B' - AEC)$. If H has rank at least r it will follow that the determination of Y as in step V, which yields $H = GEC$, will imply that $B' = AEC$, M has rank r. Conversely our remaining work in which we investigate $B' = AEC$ implies that if we make $B' = AEC$ and $G' = AEH'$ then we will have $H = GEC$.

It is necessary to investigate the relation $B' = AEC$ since we might have a case where $n = 2r$, G, H, and K_0 do not appear, and the procedure outlined above is vacuous. In this case we proceed to compute $E = (\delta_{ij})$ as usual, and in all cases where the procedure above does not determine X and Y, we continue as follows:

VII. *Compute the matrix products*

$$(\alpha_{ij}) = -EC_0, \quad (\beta_{ij}) = B' - A_0(EC_0), (\gamma_{ij}) = A_0E \quad (i,j = 1, \ldots, r). \tag{7}$$

VIII. *Solve the equations*

$$x_k[x_i(\alpha_{ij}\gamma_{kj} - \gamma_{ij}\alpha_{kj}) + (\beta_{ij}\gamma_{kj} - \delta_{ij}\alpha_{kj})] = x_i(\gamma_{ij}\beta_{kj} - \alpha_{ij}\delta_{kj}) + (\delta_{ij}\beta_{kj} - \beta_{ij}\delta_{kj}) \quad (i \neq k;\ i, j, k = 1, \ldots, r). \tag{8}$$

These equations may be used normally to solve for $r - 1$ of the unknown diagonal elements x_k as quotients of two linear functions of the remaining one. The consistency of our system will then usually determine the remaining unknown. In all cases the solution must be such that

$$|A| \neq 0. \tag{9}$$

Observe that in the exceptional case where $A_0 = C_0 = 0$ and $B = I$ the diagonal elements of A are any nonzero numbers and $C = A^{-1}$, $Y = X^{-1}$.

IX. *Solve the equations*

$$(x_i\gamma_{ij} + \delta_{ij})y_j = x_i\alpha_{ij} + \beta_{ij} \quad (i,j = 1, \ldots, r) \tag{10}$$

for the diagonal elements y_j.

Equations (10) normally yield r determinations of each y_i. These equations may also be used as checks if the diagonal elements have been determined by the use of V, VI.

Tests VIII and IX are equivalent to $B' = AEC$. For B is nonsingular and this matric equation implies (9). Then $B' = AEC(A_0 + X)E(C_0 + Y)$, $(B' - A_0EC_0) + X(-EC_0) = [(A_0 + X)E]Y$. This equation is equivalent to (10). For each value of j the equations (10) are a system of r equations in a single unknown y_j with matrix Γ_j a column of the nonsingular matrix AE, and Γ_j has rank one. Consistency of (10) demands

that the rank of the augmented matrices must be one, that is, for all $i \neq k$ and j we must have

$$(x_i\alpha_{ij} + \beta_{ij})(x_k\gamma_{kj} + \delta_{kj}) = (x_i\gamma_{ij} + \delta_{ij})(x_k\alpha_{kj} + \beta_{kj}). \tag{11}$$

These equations are another form of (8).

The various determinations of the same diagonal elements may be employed to determine them accurately in cases where there are experimental errors in the given correlation coefficients. It should be possible then to obtain data indicating clearly the correct values, and final corroboration will be obtained by factorization of the matrix M.

EXAMPLE

$$M_0 = \begin{pmatrix} 0 & 0.72 & -0.61 & 0.92 & -0.26 & 0.59 \\ 0.72 & 0 & -0.80 & 0.68 & 0.20 & 0.68 \\ -0.61 & -0.80 & 0 & -0.54 & -0.40 & -0.69 \\ 0.92 & 0.68 & -0.54 & 0 & -0.39 & 0.55 \\ -0.26 & 0.20 & -0.40 & -0.39 & 0 & 0.21 \\ 0.59 & 0.68 & -0.69 & 0.55 & 0.21 & 0 \end{pmatrix}.$$

Then

$$B = \begin{pmatrix} -0.61 & -0.80 \\ 0.92 & 0.68 \end{pmatrix}, \qquad E = \begin{pmatrix} 2.118 & 2.492 \\ -2.866 & -1.900 \end{pmatrix},$$

so that

$$GE = \begin{pmatrix} -1.124 & -1.028 \\ -0.699 & 0.178 \end{pmatrix}, \qquad GEH' = \begin{pmatrix} 0.851 & 0.210 \\ 0.210 & 0.58 \end{pmatrix}$$

This verifies our assumption that the ideal rank is two. We also compute

$$GEC_0 = \begin{pmatrix} -1.124 & -1.028 \\ -0.699 & 0.178 \end{pmatrix}\begin{pmatrix} 0 & -0.54 \\ -0.54 & 0 \end{pmatrix} = \begin{pmatrix} 0.555 & 0.607 \\ -0.096 & 0.377 \end{pmatrix}, \qquad H - GEC_0 = \begin{pmatrix} -0.955 & -0.997 \\ -0.544 & 0.173 \end{pmatrix},$$

to give $y_1 = 0.850$, $y_1 = 0.850$, $y_2 = 0.970$, $y_2 = 0.972$. Similarly

$$EH' = \begin{pmatrix} -1.819 & -0.091 \\ 1.887 & 0.933 \end{pmatrix}, \qquad A_0EH' = \begin{pmatrix} -1.359 & 0.672 \\ -1.310 & -0.066 \end{pmatrix},$$

$$G' - A_0(EH') = \begin{pmatrix} -1.619 & -0.082 \\ 1.510 & 0.746 \end{pmatrix},$$

so that $x_1 = 0.890$, $x_1 = 0.901$, $x_2 = 0.800$, $x_2 = 0.800$. The bad value of 0.901 is due to the smallness of the numbers 0.082 and 0.091, and the weighted mean

$$\frac{1.619 + 0.082}{1.819 + 0.091} = 0.891$$

indicates that the correct value is 0.89. Actually $M = FF'$, where

$$F' = \begin{pmatrix} 0.8 & 0.4 & -0.2 & 0.9 & -0.7 & 0.3 \\ 0.5 & 0.8 & -0.9 & 0.4 & 0.6 & 0.7 \end{pmatrix}.$$

[1] Cf. Thurstone, L. L., *The Vectors of Mind*, Chicago, 1935, and Lederman, W., "On a Problem Concerning Matrices with Variable Diagonal Elements," *Proc. Roy. Soc. Edin.*, Vol. 60, (1939), 17 pp.

[2] Actually $M = P\Lambda P'$ where Λ is a diagonal matrix with its last $n - r$ diagonal elements zero, P is an orthogonal matrix. Then F is the product of the first r columns of P by the respective square roots of the first r diagonal elements of Λ, that is, the square roots of the characteristic roots of M.

LAPLACE TRANSFORMS OF A CLASS OF HIGHER DIMENSIONAL VARIETIES IN A PROJECTIVE SPACE OF n DIMENSIONS

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Communicated February 15, 1944

The theory of conjugate nets and their Laplace transforms has been the object of extensive study. Its extension to r-dimensional varieties in a projective space of n dimensions, $r < n$, has so far not been very successful. The object of this note is to indicate a possible way that the theory can be extended.

Consider a projective space of n dimensions. To develop the differential geometry in the space we employ Cartan's method of moving frames, taking as projective frame the set of $n + 1$ linearly independent vectors $A, A_1, \ldots, A_n$ (each with $n + 1$ components), determined up to a common factor. The equations of infinitesimal displacement are of the form

$$\left.\begin{aligned} dA &= \omega_{00}A + \omega_1 A_1 + \ldots + \omega_n A_n \\ dA_1 &= \omega_{10}A + \omega_{11}A_1 + \ldots + \omega_{1n}A_n \\ &\vdots \\ dA_n &= \omega_{n0}A + \omega_{n1}A_1 + \ldots + \omega_{nn}A_n \end{aligned}\right\} \tag{1}$$

where the ω's are Pfaffian forms in the parameters on which depend the frames. By remarking that the exterior derivatives of the left-hand sides of (1) are zero, we get the so-called equations of structure of the space:

$$\left.\begin{aligned} \omega'_{00} &= [\omega_1\omega_{10}] + \ldots + [\omega_n\omega_{n0}] \\ \omega'_i &= [\omega_{00}\omega_i] + [\omega_1\omega_{1i}] + \ldots + [\omega_n\omega_{ni}] \\ \omega'_{ij} &= [\omega_{i0}\omega_j] + [\omega_{i1}\omega_{1j}] + \ldots + [\omega_{in}\omega_{nj}],\ i, j = 1, \ldots, n \\ \omega'_{i0} &= [\omega_{i0}\omega_{00}] + [\omega_{i1}\omega_{10}] + \ldots + [\omega_{in}\omega_{n0}] \end{aligned}\right\} \quad (2)$$

Let V_r be a variety of r dimensions in the space. We make the convention that a linear combination of the vectors of a frame represents a point whose homogeneous coördinates are its components. To each point of V_r we attach a frame such that A coincides with the point, $A_1, \ldots, A_r$ lie in the tangent r-plane, and $A_{r+1}, \ldots, A_n$ $n - r$ other points of the space. For such a family of frames over V_r we have

$$\begin{cases} \omega_{r+1} = \ldots = \omega_n = 0, \\ \omega_{i\alpha} = \sum_{j=1}^{r} g_{ij\alpha}\omega_j,\ i = 1, \ldots, r,\ \alpha = r+1, \ldots, n \end{cases} \quad (3)$$

where $g_{ij\alpha}$ are functions of parameters on the variety and are symmetric with respect to i, j. Construct the quadratic forms

$$\Phi_\alpha = \omega_1\omega_{1\alpha} + \ldots + \omega_r\omega_{r\alpha},\ \alpha = r+1, \ldots, n \quad (4)$$

and suppose q of them be linearly independent. This number q is an arithmetic invariant. We can choose the frame attached to the point A such that $\Phi_{r+1}, \ldots, \Phi_{r+q}$ are linearly independent and $\Phi_{r+q+1}, \ldots, \Phi_n$ are identically zero. We shall call the net of quadric cones of vertex A defined by the equation

$$\lambda_{r+1}\Phi_{r+1} + \ldots + \lambda_{r+q}\Phi_{r+q} = 0 \quad (5)$$

the asymptotic net at A. It generalizes the asymptotic tangents of a surface.

E. Cartan[1] studied the varieties of r dimensions whose asymptotic net is reducible to the form

$$\lambda_1\omega_1^2 + \ldots + \lambda_r\omega_r^2 = 0 \quad (6)$$

Cartan proved that such varieties depend on $r(r-1)$ arbitrary functions in two variables. It is not difficult to prove that for $r = 2$ these varieties are in general the surfaces sustaining conjugate nets.

From the last remark we can deduce a notable geometrical property of these varieties. Suppose the frames attached to the points of V_r are so chosen that the asymptotic net has the equation (6). Then the lines AA_i, $i = 1, \ldots, r$, are invariantly related to the variety. They define on the variety r families of curves such that through every point passes one and only one curve of each family. If the tangent r-plane is displaced along a curve of the ith family, its intersection with a neighboring tangent

r-plane is the tangent $(r-1)$-plane $AA_1 \ldots A_{i-1}A_{i+1} \ldots A_r$. This is clearly a generalization of the well-known property of conjugate nets.

It turns out that this property is characteristic to the varieties under consideration. In fact, suppose that a variety of r dimensions possesses r families of curves such that when the tangent r-plane is displaced along a curve of one family, its intersection with a neighboring tangent r-plane is the tangent $(r-1)$-plane tangent to the other $r-1$ curves. Then, under a certain generality assumption, the variety has the property that its asymptotic net is reducible to the equation (6). The generality assumption has to be made, because there are some degenerate cases, which are not interesting. A simple and reasonable assumption of this kind is that the osculating planes of the variety are of $2r$ dimensions.

The above results can be described as a geometrical interpretation of the varieties studied by Cartan. To define for such varieties a kind of transformation generalizing the transformation of Laplace, we proceed as follows:

Let V_r be such a variety whose r families of curves have at the point A the tangents AA_i, $i = 1, \ldots, r$. Then the following theorem can be proved: On each tangent AA_i there exist $r-1$ points A_{ij} $(j \neq i)$ such that when the s-plane $AA_{i_1} \ldots A_{i_s}$ is displaced along AA_i, $j \neq i_1, \ldots, i_s$, its intersection with a neighboring s-plane is the $(s-1)$-plane $A_{i_1 j} \ldots A_{i_s j}$.

Associated to each point A we have therefore $r(r-1)$ points A_{ij}. When A describes our variety, each point A_{ij} describes a variety of dimension $\leqq r$. It can be proved that if the locus of A_{ij} is of dimension r, it also has the property described above. It is natural to call these varieties the Laplace transforms of V_r, there being altogether $r(r-1)$ Laplace transforms. The Laplace transforms of a Laplace transform of V_r will consist of V_r, some of the Laplace transforms of V_r, and some new varieties. There is a great number of interesting problems which generalize the classical theory of periodic Laplace sequences. The problems seem to be particularly fascinating, because it is probable to expect some results which are not direct generalizations of the theory of surfaces.

The proofs of the above results offer no difficulty and are therefore omitted. For long time there was the opinion that the projective differential geometry of r-dimensional varieties in a space of n dimensions has serious analytical difficulties. We wish to point out here that Cartan's method of moving frames offers a workable scheme which, at least theoretically, can push to an end the problems which are of geometrical interest.

[1] Cartan, E., "Sur les variétés de courbure constante d'un espace euclidien ou non-euclidien," *Bull. Soc. math. France*, **47**, 125–160 (1919); **48**, 132–208 (1920).